Human CD8$^+$/CD45RA$^-$ Memory T Cell Subset Column Kit

Catalog Number: HCD45

Provided in Each Kit

4 - CD8$^+$/CD45RA$^-$ Subset Columns

4 - 1 mL vials of Monoclonal Antibody Cocktail

1 - 30 mL of 10X Column Buffer Concentrate

Store all reagents at 2 - 8° C.

Intended Use

Designed to isolate sub-populations of CD8$^+$/CD45RA$^-$ human T cells via high affinity negative selection. The resulting column eluate is a highly enriched T cell subset population with minimal contamination of either CD45RA$^+$ or CD4$^+$ cells.

Principle of Selection

Leukocyte suspensions are incubated with a mixture of monoclonal antibodies and then loaded onto T Cell Subset Columns. B cells and non-selected T cell subsets bind to anti-Ig coated glass beads via F(ab)-surface immunoglobulin (Ig) interactions, while monocytes bind to Ig coated glass beads via Fc interactions. The resulting column eluate contains a highly enriched T cell subset population with virtually no B cells, monocytes, or non-selected T cell subsets. Recovery of CD8$^+$/CD45RA$^-$ cells from all available CD8$^+$/CD45RA$^-$ cells loaded ranged between 4% and 14% and the purity (CD8$^+$/CD45RA$^-$) of recovered cells ranged between 80% and 90%, with no detectable CD8$^+$/CD45RA$^+$ or CD3$^+$/CD4$^+$ cells among recovered cells.

Procedure for Use of Columns

Reagent Preparation

1) For each column to be used, prepare 75 mL of 1X Column Buffer by mixing 7.5 mL of 10X Column Buffer Concentrate with 67.5 mL of sterile distilled water *(see Technical Notes).*

Sample Preparation

2) Isolate leukocytes by standard density gradient separation. Erythrocytes must be removed by use of a hypotonic lysing reagent. R&D Systems' Human Erythrocyte Lysing Kit (Cat. # WL1000) is suitable for this procedure. *See reverse for lysing instructions*.

T Cell Subset Purification

3) Mix 2 x 10^8 leukocytes in 1 mL of sterile 1X Column Buffer with 1 vial of Monoclonal Antibody Cocktail (1 mL). Gently mix and then incubate at room temperature for 15 minutes. *During the incubation, begin washing the column with 1X Column Buffer as described in step 6.*

4) Wash the cells twice with 10 mL of 1X Column Buffer, centrifuging the cells at 300 x g for 10 minutes and decanting the supernatant after each wash.

5) Resuspend the final cell pellet in 2 mL of 1X Column Buffer.

T Cell Subset Column Preparation

6) Place the column in a column rack or ring stand. Remove the top cap of the column first to avoid drawing air into the bottom of the column. Next, remove the bottom cap. Allow the column fluid to drain into a waste receptacle. Rinse the outside tip of the column with a 70% ethanol solution during this time to ensure sterile cell processing.

7) Wash the column with 10 mL of 1X Column Buffer and allow the eluate to drain into a waste receptacle. The column is now ready for the addition of cells.

8) Replace the waste receptacle with a sterile 15 mL conical centrifuge tube.

CD8/CD45 T Cell Selection

9) Apply the antibody treated cells to a Subset Column and allow them to enter into the column. *The cells will displace some of the buffer in the column, which can be collected.*

10) Incubate the cells, now suspended in the column, at room temperature for 10 minutes.

11) After the incubation step, elute the cells from the column using a total of 12 mL of 1X Column Buffer. Collect the eluate until it appears clear.

12) Centrifuge the collected cells at 250 x g for 5 minutes. Decant the supernatant and resuspend the cells in the appropriate buffer or culture medium. The cells are ready to count and use in the desired applications.

References

1. Wigzell, H. (1976) in *In Vitro Methods in Cell-Mediated and Tumor Immunity*. B.R. Bloom and J.R. David eds. Academic Press, New York. p. 245
2. Binz, H. and H. Wigzell (1975) J. Exp. Med. **142**:1231

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Technical Notes:

In order to best determine column performance, we advise that users retain a small portion of the starting cell population. Following cell selection with the column, it may then be possible to perform immunophenotyping analysis on both starting and eluted cells. This information, when combined with the actual number of cells loaded and recovered, can then be used to calculate the percentage recovery of the target cell population.

Some of the salts in the 10X Column Buffer Concentrate may precipitate after storage at 2 - 8° C. Should this be the case, do not carry out the 1:10 buffer dilution (as indicated in step 1) until all salts are in solution. This may be achieved by warming the 10X Column Buffer Concentrate bottle in a 37° C water bath for 5 - 10 mintues. Once there is no longer evidence of precipitate, the 10X Column Buffer Concentrate may be diluted 1:10 to prepare the 1X Column Buffer necessary for column processing.

Lysing of Erythrocytes from Cell Preparations:

To remove red blood cells (RBC) from the mononuclear cell population to be loaded onto the T-cell column we suggest the following:

- Process cells on a density gradient (*e.g.* Ficoll Hypaque) to enrich for mononuclear cells.
- Recover the “buffy coat”, containing the mononuclear cells, and wash the cells 2 times with excess PBS to remove any contaminating separation media. This can be done by centrifuging the cells at 200 x g for 10 minutes.
- After the second wash step, resuspend the cell pellet in H-lyse Buffer contained in R&D Systems’ Human Erythrocyte Lysing Kit (Cat. # WL1000). *Dilute the 10X H-lyse Buffer to 1X before using. 10 mL of 1X H-lyse Buffer per 250 million cells is recommended.*
- Incubate the cells for 10 minutes at room temperature. Add sufficient 1X Wash Buffer contained in the Human Erythrocyte Lysing Kit to fill the tube. *Dilute the 10X Wash Buffer to 1X before using.*
- Centrifuge the cells at 200 x g for 10 minutes. Resuspend the cells in a small volume of 1X Column Buffer (contained in the T Cell Subset Column Kit).
- Perform a cell count and adjust the cell concentration to 1 - 2 x 10^8 cells/mL using 1X Column Buffer (contained in the T Cell Subset Column Kit).
- Continue cell selection by proceeding to step 3 on the opposite page.